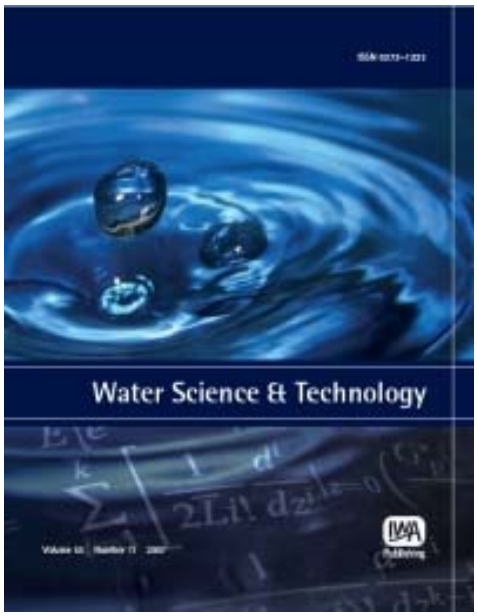

Obtention of plant peroxidase and its potential for the decolorization of the reactive dye Remazol Turquoise G 133%

Maria Cristina Silva, Juliana Arriel Torres, Angelita Duarte Corrêa, Allana Maria Bernardes Junqueira, Maria Teresa Pessoa Amorim and Custódio Donizete dos Santos

ABSTRACT

Peroxidases can be used in the decolorization process. There is a growing interest for new sources of this enzyme and for obtaining economically viable processes. In this work, a low-cost vegetable peroxidase extraction process is proposed; the resulting enzyme is characterized to determine its optimum pH, temperature, and stability conditions, and it is then applied in the decolorization of reactive dye Remazol Turquoise G 133%. The turnip peroxidase (TP) was utilized as an enzymatic source. This enzyme exhibited maximum activity at pH 7.0, and it was active in the temperature range of 30 to 50 °C, which favors its use in industrial processes. Acetone was the most efficient solvent to induce precipitation. The removal of Remazol Turquoise G 133% was 56.0% complete after 50 min, while 41.0% of the same dye was removed with the commercial horseradish peroxidase enzyme in 50 min. TP presents potential as a viable alternative in the decolorization of textile wastewaters.

Key words | decolorization, environmental biocatalysis, peroxidase, textile dyes, turnip

Maria Cristina Silva
Juliana Arriel Torres (corresponding author)
Angelita Duarte Corrêa
Allana Maria Bernardes Junqueira
Custódio Donizete dos Santos
Department of Chemistry,
Federal University of Lavras,
37200-000, Lavras, MG,
Brazil
E-mail: *julianaarriel@hotmail.com*

Maria Teresa Pessoa Amorim
Department of Textile Engineering,
University of Minho,
4800-058 Guimarães,
Portugal

INTRODUCTION

The textile industry plays an important role in most countries, being one of the traditional industrial segments. However, textile processes consume large quantities of water, chemical products and synthetic dyes, and they generate large volumes of wastewater that contain a high organic load.

In general, it is believed that approximately 20% of the dye load is lost in the dyeing residues during textile processing, which represents one of the great environmental problems faced by the sector (Guarantini & Zanoni 2000). Considering that more than 700 thousand tons of dyes and pigments are produced annually in the world and that Brazil is responsible for the consumption of about 2.6% (Zanoni & Carneiro 2001), the harmful effect of the liberation of dyes in the environment becomes quite significant.

When not treated appropriately, and when discharged in natural waters, the wastewaters originating from the dye industry or processes involving the dyeing of textile fibers can modify the ecosystem, reducing the transparency of the water and the penetration of solar radiation, which can reduce photosynthetic activity and the solubility of gases.

In general, the textile industry uses treatment systems based on physico-chemical and biological systems, which in many cases are incompatible with the characteristics of the wastewater generated, mainly with respect to the removal of the color. From this point of view, the study of new treatment alternatives is essential.

The peroxidases (donor H_2O_2 oxidoreductase, E.C. 1.11.1.7) are enzymes that catalyze the reduction of hydrogen peroxide or another organic peroxide when an electron donor is oxidized. The reaction occurs in multiple stages, as shown below:

$$\text{Peroxidase} + H_2O_2 \rightarrow \text{Compound I} + H_2O \quad (1)$$

$$\text{Compound I} + AH_2 \rightarrow \text{Compound II} + A^{\bullet} \quad (2)$$

doi: 10.2166/wst.2012.892

$$\text{Compound II} + AH_2 \rightarrow \text{Peroxidase} + AH^{\bullet} + H_2O \quad (3)$$

In the first stage of the catalytic process, the reaction of the active site with hydrogen peroxide occurs. The hydrogen peroxide is reduced, producing water and compound I, a reactive intermediate that has a higher oxidation state than the native enzyme. In the second stage of the reaction, compound I oxidizes a substrate molecule (AH_2), generating a substrate radical and compound II. Finally, a second substrate molecule reduces compound II, returning the enzyme to its initial form (Hiner *et al.* 2001).

This class of enzymes is widely distributed in the plant and animal kingdoms, and it is found in microorganisms, plants and mammals (Veitch 2004).

Peroxidase has been used in biotechnology and several other areas of science for the establishment of clinical diagnoses, in the evaluation of pathological processes, in food quality analysis, in the construction of biosensors for qualitative and quantitative analysis of pharmaceutical and cosmetic formulas and in paper and cellulose manufacturing. In the environment, the water pollution index can be evaluated through tests using peroxidases. Furthermore, these enzymes can be used in the decolorization process to decrease textile industry pollutant residues (Maciel *et al.* 2006, 2007).

Besides the use in decolorization processes, peroxidases can be used for the removal of phenolic compounds by oxidizing them to phenoxy radicals, which then react to create less soluble hydrophobic polymeric products (Li *et al.* 2011). horseradish peroxidase has been utilized for the removal of halogenated phenols (Meizler *et al.* 2011) and pentachlorophenol (Li *et al.* 2011). Other peroxidases, such as peroxidases from *Allium sativum, Ipomoea batatas, Raphanus sativus*, *Sorghum bicolor* and soybean peroxidase have also been applied to phenol removal (Al-Ansari *et al.* 2010; Diao *et al.* 2011).

The limitation on the use on plant peroxidases is the low yield and high production cost compared with bacterial or fungal enzymes. However, the production cost of these enzymes can be reduced by optimizing the extraction conditions and by using plant material with high enzymatic activity that can be directly applied with the same efficiency as the purified enzyme (Dec & Bollag 1994).

The peroxidases occur in the soluble and bonded form, associated with the cell walls of plant cells and probably associated with certain organelles (Khan & Robinson 1994). The soluble fraction can be extracted with water or with a low ionic strength buffer, the ionically bonded fraction with a high ionic strength buffer containing NaCl or $CaCl_2$, and the covalently bonded form using cellulolytic or pectinolytic enzymes (Vámos-Vigyázó 1981).

The use of additives is advisable in the extraction of plant enzymes. The chemical compounds that are usually used include buffers, whose function is the protection of the enzymes from acids liberated from the vacuoles after the rupture of the cell and the desorption of the enzyme bonded to the cell wall; PEG and PVP (polyvinylpolypyrrolidone), which protect against inactivation by phenols and their oxidation products and EDTA as a chelator (Doonan 1996).

The most commonly used commercial peroxidase source is horseradish (horseradish peroxidase), which is usually cultivated and harvested in cold climate countries (Maciel *et al.* 2006). Several reports in the literature point to the use of horseradish peroxidase in the degradation of several dyes: (1) Mohan *et al.* (2005) obtained 79% degradation of acid black dye with the horseradish peroxidase immobilized in acrylamide gel, and 67% with the free enzyme; (2) Ferreira-Leitao *et al.* (2003) studied the degradation of methylene blue dye by horseradish peroxidase. In this work, only 4.7% of the dye remained in solution, for a 1:10 proportion of dye/H_2O_2; and (3) Bhunia *et al.* (2001) showed that horseradish peroxidase can be effective in the degradation and precipitation of important industrial azo dyes.

Due to the widespread use of peroxidases, mainly as an environmental biocatalyst, there is a growing interest in new sources of this enzyme.

In this context, the objectives of this work included obtaining a new vegetable source rich in peroxidase; the determination of the optimum pH, temperature and stability conditions of this enzyme, which are important parameters to evaluate the potential application of the enzyme in industrial processes; the evaluation of its potential in the degradation of the reactive dye Remazol Turquoise G 133%; and a comparison with the degradation potential of the commercial horseradish peroxidase enzyme.

METHODS

Vegetables, commercial enzyme and dye

The following vegetables were used to obtain the peroxidase: turnip (*Brassica campestre* ssp. *rapifera*), radish (*Raphanus sativus)*, zucchini squash (*Curcubita pepo*), gilo (*Solanum gilo* Raddi) and sweet potato (*Ipomoea*

potatoes (L.) *Lam.*). The fruits of the squash and gilo, the roots of the turnip and radish, the tuber of the sweet potato, and the leaves and the peels of all the vegetables were used.

The horseradish peroxidase enzyme (HPR II) was acquired from Sigma Aldrich and used in aqueous solution (30 mg of the commercial enzyme in 20 mL of 0.05 mol L^{-1} phosphate buffer, pH 6.5). After the preparation, the enzyme was stored at an average temperature of 4 °C.

Statistical analysis

The variance analysis of the results, when applicable, was made using SISVAR software (Ferreira 2003) and, when significant, the Scott–Knott test, to 5% of probability, was applied for comparison of the averages.

Obtention of the raw enzymatic extract

The fruits, roots and tubers of the vegetables were properly washed in running water and distilled water. Soon afterwards, 25 g of the peeled vegetable tissue was cut up and homogenized in a blender with 100 mL of 0.05 mol L^{-1} phosphate buffer (pH 6.5) for 30 s. The homogenate was filtered through organza cloth and centrifuged at 10,000*g* for 15 min, at 4 °C. The supernatant solution was stored at 4 °C and used as the enzymatic source of the peroxidase (Fatibello-Filho & Vieira 2002). The peels and leaves of the vegetables were washed in running water and distilled, cut into small uniform pieces, and subjected to the same extraction procedure described previously.

The experimental design used was completely randomized with 15 treatments and three repetitions of three vegetables.

Determination of the enzymatic activity

The activity was determined according to Khan & Robinson (1994), using the following reaction medium: 1.5 mL guaiacol (Vetec; 97%) 1% (v/v), 0.4 mL of H_2O_2 (Vetec, PA, 0.3% (v/v)), 0.1 mL enzyme (maintained in an ice bath) and 1.2 mL of 0.05 mol L^{-1} phosphate buffer (pH 6.5). The reaction was incubated for 5 min at 30 °C in a Spectrovision spectrophotometer that was coupled to a thermostatic bath.

One unit of peroxidase activity represents the oxidation of 1 μmol of guaiacol in 1 min in the assay conditions, and it was calculated using data relative to the linear portion of the curve.

Influence of additives on the enzymatic activity

The additives NaCl (0.2 mol L^{-1}), KCl (0.2 mol L^{-1}), PEG 4000 (polyethylene glycol, 2% (p/v)), PVP (insoluble polyvinylpyrrolidone, 2% (p/v)) and EDTA (10 mmol L^{-1}) were added separately into the extrator solution to evaluate their influence on the enzymatic activity (Holschuh 2000). The control was the activity in the absence of any additives.

The experimental design we adopted was completely randomized with five treatments and three repetitions.

Influence of pH on enzymatic activity and thermal stability

The optimum pH was determined by varying the pH of the buffer solutions from 2.0 to 9.0 in intervals of one pH unit. The buffers used were citrate buffer (0.1 mol L^{-1}, pH 2.0 to 6.0) and Tris-HCl buffer (0.1 mol L^{-1}, pH 7.0 to 9.0).

The experimental design that was adopted was completely randomized with eight treatments and three repetitions.

The thermal stability of the peroxidase was evaluated by incubating the enzyme for 1, 2 and 4 h, and by varying the temperature from 30 to 90 °C at intervals of 10 °C. After the thermal treatment, the samples were cooled in an ice bath, and the residual activity was determined. As a control, the raw enzymatic extract activity without thermal treatment was determined. Three repetitions for each treatment were carried out.

Precipitation of proteins

Precipitation by acetone

Cold acetone was added to the raw enzymatic extract until it reached a concentration of 65% (v/v). After incubating for 12 to 14 h, at −18 °C, the homogenate was centrifuged at 11,000*g* for 15 min at 4 °C. The supernatant was discarded. The precipitate containing the peroxidase was submitted to the removal of the acetone by evaporation in an ice bath for 3 h. The precipitate was re-suspended in 10 mL of sodium phosphate buffer (pH 6.5), and the resulting suspension was used for the determination of the enzymatic activity. The procedure was repeated three times.

Precipitation by ammonium sulfate

The precipitation by ammonium sulfate was performed according to literature precedent (Zeraik *et al.* 2008), with some modifications. In the first stage, solid ammonium

sulfate was added to the extract, so as to obtain a 40% saturation. At this point, a clarification of the extract solution is observed, originating from the precipitation of the cytoplasmic and nuclear materials and/or proteins present. That solution was maintained at 4 °C for 20 h and centrifuged at 8,000*g* for 10 min, at 4 °C. The precipitate was discarded, and more solid ammonium sulfate was added to the supernatant to reach an 85% saturation. The solution was maintained at 4 °C for an additional 20 h. The supernatant was separated from the precipitate by centrifugation at 8,000*g* for 10 min at 4 °C, and at this stage, the supernatant was discarded, and the precipitate was resuspended in 5 mL of pH 6.5 sodium phosphate buffer.

The resulting suspension was dialyzed against a pH 6.5 sodium phosphate buffer for 24 h (32 mm *benzoylated dialysis* tubing with a cutoff range of 2 kDa, five daily changes, in a refrigerator and subjected to magnetic agitation) for the removal of the ammonium sulfate. The resulting suspension was submitted for enzymatic activity determination. The procedure was conducted in triplicate.

Decolorization assays

Based on the methodology described by Khan & Robinson (1994) with modifications, the enzymatic oxidation reactions of the textile dyes were conducted at 30 °C in 1.2 mL phosphate buffer (0.05 mol L^{-1}, pH 7.0) containing 0.4 mL H_2O_2, 1.5 mL Remazol Turquoise G 133%, and 0.1 mL of enzymatic solution. The final volume of reaction medium was 3.2 mL. The H_2O_2, enzyme and dye concentrations at middle were 100 μmol L^{-1}, 20.3 U mL^{-1} and 50 mg L^{-1}, respectively.

The plant enzymatic extract that is considered most appropriate for use in the oxidation of the dyes (which presents higher enzymatic activity) was treated with acetone to induce precipitation as described, and used in the decolorization assays.

The reaction mixture was incubated in a spectrophotometer that was coupled to a thermostatic bath, and the absorbance of the dye was measured at different times during the experiments. Monitoring of the oxidation was done at 624 nm, the maximum wavelength for Remazol Turquoise G was 133%. The calculation to determine the percentage of color removal of the dyes was done according to the equation:

$$\frac{\text{absorbancy}_{\text{initial}} - \text{absorbancy}_{\text{final}}}{\text{absorbancy}_{\text{initial}}} \times 100$$

To evaluate the dye adsorption by the enzymatic extract, the reaction medium containing 1.6 mL phosphate buffer (0.05 mol L^{-1} pH 7.0), 1.5 mL of the Remazol Turquoise G 133% dye (50 mg L^{-1}) and 0.1 mL of the enzymatic extract (12.18 U mL^{-1}) was incubated in a spectrophotometer at 30 °C for 2 h and the dye removal was analyzed at 624 nm.

RESULTS AND DISCUSSION

Obtention of a vegetal source rich in peroxidase

Among the vegetable sources that were analyzed, the turnip peel (*Brassica campestre* ssp. *rapifera*) provided higher enzymatic activity, while the turnip (*Brassica campestre* ssp. *rapifera*) and radish leaves (*Armoracia rusticana*) presented lower activity (Table 1). It was also observed that the turnip root constitutes a rich peroxidase source; therefore, the turnip was adopted (peel and root) as the main source of the enzyme. All of the subsequent assays were conducted using this enzymatic source.

Influence of additives on peroxidase activity

The influence of the additives on the peroxidase activity was investigated. There was significant variation in the activity of the peroxidase obtained from the turnip extract (PET) under the influence of additives (Table 2). The extractor solution containing NaCl (0.2 mol L^{-1}) induced a higher increase of the enzymatic activity, followed by KCl (0.2 mol L^{-1}) and EDTA (10 mmol L^{-1}), while 2% (p/v) PEG and 2% (p/v) PVP resulted in a decrease in the activity.

Table 1 | Peroxidase activity of raw extracts from different plant tissues

Sample	Plant tissues	Activity (U mL^{-1})*	Activity (U g^{-1} plant tissue)
Zucchini squash (*Curcubita pepo*)	Fruit	0.223 c	0.892
	Peel	1.253 g	5.012
	Leaves	0.845 e	3.380
Sweet potato (*Ipomoea potatoes* (L.) *Lam.*)	Tuber	0.105 b	0.420
	Peel	0.812 e	3.248
	Leaves	0.316 d	1.264
Gilo (*Solanum gilo* Raddi)	Fruit	0.275 d	1.100
	Peel	0.159 c	0.636
	Leaves	0.189 c	0.756
Turnip (*Brassica campestre* ssp. *rapifera*)	Root	1.080 f	4.320
	Peel	1.487 h	5.948
	Leaves	0.024 a	0.096
Radish (*Armoracia rusticana*)	Root	0.225 c	0.900
	Peel	1.039 f	4.156

*Averages followed by the same letter in column do not differ among themselves by Scott–Knott test at 5% probability.

Table 2 | Influence of additives on the activity of peroxidase, obtained from the raw extract of radish*

Additives	Activity (U mL^{-1})*	Activity (U g^{-1} plant tissue)
PEG (2%)	0.248 a	0.992
PVP (2%)	0.262 a	1.048
Control (without additives)	0.330 b	1.320
EDTA (10 mmol L^{-1})	0.398 c	1.592
KCl (0.2 mol L^{-1})	0.473 d	1.892
NaCl (0.2 mol L^{-1})	0.776 e	3.104

*Averages followed by the same letter in the column do not differ among themselves by Scott–Knott test at 5% probability.

Several studies describe a significant improvement in the activity and stability of enzymes when the extraction procedure is accomplished in the presence of additives. Additives have been used for protection from enzyme inactivation, retention of the water layer around the biocatalyst and as enzyme molecule dispersers and mass transport facilitators. The interaction of the additive with the enzyme can present antagonistic behavior; that is, the interaction can present a negative effect in the reaction of interest, decreasing the efficiency of enzyme, as was observed for the 2% (p/v) PEG and 2% (p/v) PVP additives. Soares *et al.* (2003) affirms that not all of the additives are efficient stabilizers, and the influence of the additive on the enzymatic activity still has not been totally clarified.

Influence of pH on enzyme activity and thermal stability

The PET activity showed significant variation as a function of the pH. PET presented high activity at pH 7.0 (Figure 1).

In acidic pH, the peroxidase presented an activity decrease. This activity decrease might have occurred mainly by ionic alterations of the enzyme that alter the form of the enzyme and consequently the active site. The activity decrease can also be observed at pH 9.

The pH for the maximum peroxidase activity varies with the enzyme source, the isoenzyme composition, the donor substrate, and the buffer used for the analysis (Vámos-Vigyázó 1981).

pH values in the range of 5.7 to 5.9 were found for the carom peroxidase, using guaiacol as the substrate (Holschuh 2000). The peroxidase of the leaves of *Copaifera langsdorffii* (Diesel Tree) presented high activity in the pH range of 5.5 to 6.0 (Maciel *et al.* 2006).

The study of the effect of temperature on the PET stability showed that the enzyme is thermostable up to 40 °C after 1, 2 and 4 h of incubation (Figure 2). At 50 °C, a small activity

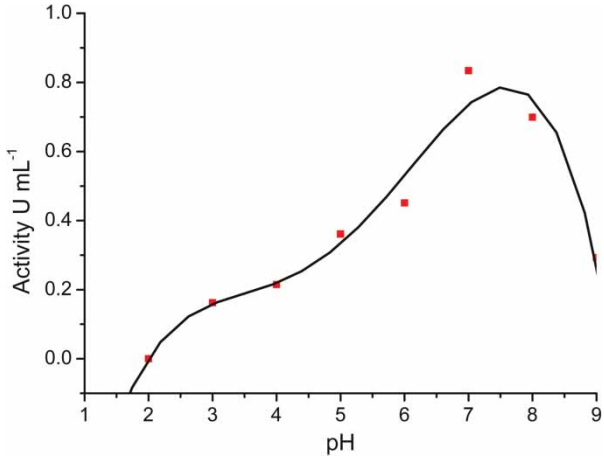

Figure 1 | Effect of pH on peroxidase activity obtained from the crude extract of turnip.

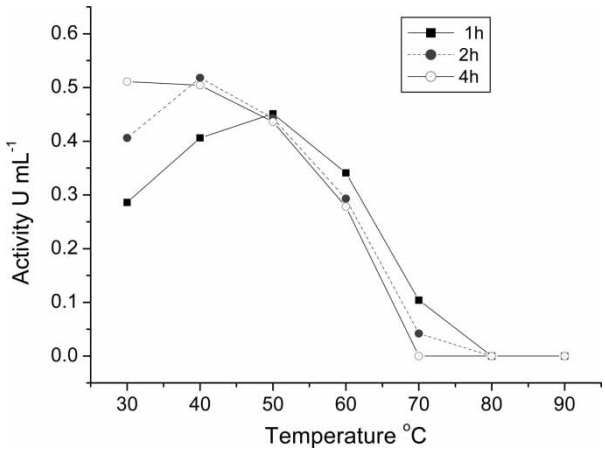

Figure 2 | Influence of temperature and incubation time on the stability of peroxidase obtained from the crude extract of turnip.

decrease was observed after 2 and 4 h of incubation. The enzymatic activity was lost completely at temperatures above 70 °C.

The peroxidase stability is very important for its use in various areas of the sciences. The higher the stability and enzymatic activity, the better the enzyme application capacity in diverse methods such as application as biocatalysts (Maciel *et al.* 2007). According to the results obtained in a temperature range of 30 to 50 °C, the resulting enzyme did not present a significant decrease in the enzymatic activity, which favors its use in industrial processes.

Precipitation of the proteins

The most efficient precipitating agent was acetone, which led to a 94.48 ± 0.62% recovery in terms of the enzyme activity, while the ammonium sulfate gave only 64.85 ± 6.49% recovery of activity. Besides providing high recovery, the precipitation

with acetone does not require dialysis, thus reducing the duration and cost of the process, which yields the enzyme through an economically simple and viable process. Therefore, precipitation with acetone to yield the enzyme was performed.

Decolorization of Remazol Turquoise G 133% enzymatic dye catalyzed by horseradish peroxidase (HPR) and turnip peroxidase

The Remazol Turquoise G 133% dye is widely used by textile industries. It belongs to the class of reactive dyes and contains a monofunctional group and vinyl sulfone as the reactive group.

The decolorization of the dye in aqueous solution catalyzed by horseradish peroxidase (22 U mL^{-1}) was 41% after 50 min in the presence of the enzyme, while when catalyzed by the peroxidase of *Brassica campestre* ssp. *rapifera* (20.3 U mL^{-1}), 56% of the dye was degraded under the same assay conditions (Figure 3).

Similar results were found by Souza *et al.* (2007) who obtained 59% of Remazol Turquoise G 133% dye decolorization in aqueous solution with HPR under the following conditions: a dye concentration of 100 mg mL^{-1}, 29.85 U mL^{-1} of HPR, 2 μmol L^{-1} of H_2O_2 and a pH between 4.0 and 5.0 at 30 °C.

There was no adsorption of the dye by the enzymatic extract because in the absence of hydrogen peroxide, there was no dye reduction. This situation indicates that the decolorization occurs exclusively as a function of the catalytic activity of the enzyme.

Considering the kinetics of biodegradation of dye Remazol Turquoise G 133% by turnip peroxidase and horseradish peroxidase enzymes, the pseudo-constant kinetics were calculated for both enzymatic reactions, in accordance with Barreto and coworkers (2011).

Data obtained (Table 3) suggested that both enzymatic reactions (Table 1), follow a pseudo-second-order kinetics. The results also showed that the reaction catalyzed by turnip peroxidase presented the higher pseudo-constant kinetic when compared with horseradish peroxidase.

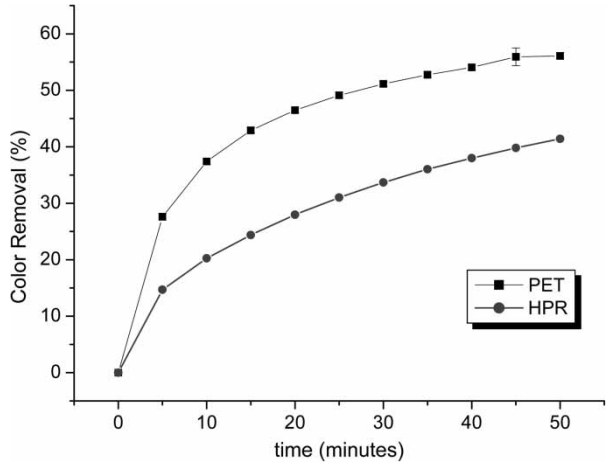

Figure 3 | Removal of the Remazol Turquoise G 133% dye catalyzed by the commercial enzyme horseradish peroxidase (HPR) and by peroxidase of *Brassica campestris* ssp. rapifera (PET).

Similar results were found by Barreto and coworkers (2011). The degradation of Procion Yellow and Procion Blue dyes by *Ganoderma* sp. resulted in the following pseudo-constant kinetics: 0.31 and 0.43×10^{-3} h^{-1}, respectively.

The peroxidase of *Brassica campestre* ssp. *rapifera* turnip peroxidase presented a degradation potential for this dye that was superior to HPR without the need for purification. The elimination of purification steps decreases the cost to obtain the enzyme, enabling it to be used as an economically viable alternative in the treatment of textile wastewaters.

Many treatments can be efficient in the decolorization, but it is essential to know if there is formation of toxic products during the process. Previous studies showed that there was an increase in toxicity after enzymatic decolorization of Remazol Turquoise G 133% by *horseradish peroxidase* horseradish peroxidase (Forgiarini 2006). These data suggest the formation of degradation products more toxic than the parental molecule. The increased toxicity may also be ascribed to the release of the Cu^{2+} ions of the dye structure (Forgiarini 2006). Therefore, this fact shows the importance of toxicological evaluation after enzymatic treatment. In this case, when the metabolites formed are

Table 3 | Kinetic parameters calculated for biodegradation of the Remazol Turquoise G 133% dye by TP and HRP

Enzyme	Pseudo-order	Equation	R^2	$K/10^{-3}$ h^{-1}
Turnip peroxidase (TP)	Zero	$y = -15.957 - 0.2805x$	0.864	280.5
	Pseudo-first order	$y = -0.3697 - 0.0103x$	0.909	10.3
	Pseudo-second order	$y = 0.0084 + 0.0004x$	0.946	0.4
Horseradish peroxidase (HRP)	Zero	$y = -7.491 - 0.2861x$	0.964	286.1
	Pseudo-first order	$y = -0.1496 - 0.0082x$	0.9812	8.2
	Pseudo-second order	$y = 0.0028 + 0.0002x$	0.9928	0.2

more toxic than the parent molecule, the enzymatic treatment should be considered as pre-treatment.

CONCLUSIONS

The enzyme obtained presented optimum activity at pH 7.0 and proved thermally stable. The extraction solution containing NaCl 0.2 mol L^{-1} provided an increase in enzyme activity.

The turnip peroxidase was capable of removing up to 56% of the Remazol Turquoise G 133% dye in aqueous solution after 50 min of reaction. While the decolorization obtained for horseradish peroxidase was 41% under the same conditions.

The efficiency of turnip peroxidase in the oxidation of the dye was comparable with horseradish peroxidase, a commercial enzyme generally utilized in discoloration processes.

REFERENCES

Al-Ansari, M. M., Modaressi, K., Taylor, K. E., Bewtra, J. K. & Biswas, N. 2010 Soybean peroxidase-catalyzed oxidative polymerization of phenols in coal-tar wastewater: comparison of additives. *Environmental Engineering Science* **27**, 967–975.

Barreto, W. J., Bernardino, N. D., Afonso, R. & Doi, S. M. O. 2011 Biodegradation of a mixture of textile dyes using the fungus *Ganoderma* sp: a kinetic study. *Química Nova* **34**, 568–572.

Bhunia, A., Durani, S. & Wangikar, P. P. 2001 Horseradish peroxidase catalyzed degradation of industrially important dyes. *Biotechnology and Bioengineering* **72**, 562–567.

Dec, J. & Bollag, J. M. 1994 Use of plant material for the descontamination of water polluted with phenols. *Biotechnology and Bioengineering* **44**, 1132–1139.

Diao, M., Ouédraogo, N., Baba-Moussa, L., Savadogo, P. W., N'Guessan, A. G., Bassolé, I. H. N. & Dicko, M. H. 2011 Biodepollution of wastewater containing phenolic compounds from leather industry by plant peroxidases. *Biodegradation* **22**, 389–396.

Doonan, S. 1996 *Protein Purification Protocols*, vol. 59, 405 pp. Humana Press, Towota.

Fatibello-Filho, O. & Vieira, I. C. 2002 Analytical use of raw extract from vegetables tissue such as enzyme source. *Química Nova* **25**, 455–464.

Ferreira, D. F. 2002 Statistical analysis through Sisvar (System for analysis of variance) for Windows 4.0. In: *Annual Meeting of Region of the Brazilian Society International Biometrics*, 45. São Carlos. Abstracts São Carlos: UFSCar, 2000. pp. 255–258.

Ferreira, D. F. 2003 SISVAR: version 4.6 (build 6.1) software. Lavras: DEX/UFLA. Available from: http://www.dex.ufla.br/danielff/dff02.htm (accessed 15 Dec 2010).

Ferreira-Leitao, V. S., da Silva, J. G. & Bon, E. P. S. 2003 Methylene blue and azure B oxidation by horseradish peroxidase: a comparative 19 evaluation of class II and class III peroxidases. *Applied 443 Catalysis B444 Environmental* **42**, 213–221.

Forgiarini, E. 2006 *Degradation of Dyes and Textile Wastewaters by Horseradish Peroxidase (HPR) Enzyme.* Master Thesis, Federal University of Santa Catarina, Brazil.

Guarantini, C. C. I. & Zanoni, M. V. 2000 Textile dyes. *Química Nova* **23**, 71–75.

Hiner, A. N. P., Hernandez-Ruiz, J., Williams, G. A., Arnao, M. B., Garcia-Canovas, F. & Acosta, M. 2001 Catalase-like oxygen production by horseradish peroxidase must predominantly be an enzyme-catalyzed reaction. *Archives of Biochemistry and Biophysics* **392**, 295–302.

Holschuh, H. J. 2000 *Isolation, Purification and Biochemical Characterization of Peroxidase Carambola (Averrhoa carambola, L.)*. PhD Thesis, State University of Campinas, Brazil.

Khan, A. A. & Robinson, D. S. 1994 Hydrogen donor specifcity of mango isoperoxidases. *Food Chemistry* **49**, 407–410.

Li, H., Li, Y., Cao, H., Li, X. & Zhang, Y. 2011 Degradation of pentachlorophenol by a novel peroxidase-catalyzed process in the presence of reduced nicotinamide adenine dinucleotide. *Chemosphere* **83**, 124–30.

Maciel, H. P. F., Gouvêa, C. M. C. P. & Pastore, G. M. 2006 Obtention of a new source of peroxidase from Copaífera langsdorffii Desf. with high activity. *Ciência e Tecnologia de Alimentos* **26**, 735–739.

Maciel, H. P. F. G., Gouvêa, C. M. C. P. & Pastore, G. M. 2007 Extraction and partial characterization of peroxidase from *Copaifera langsdorffii* Desf. Leaves. *Ciência e Tecnologia de Alimentos* **27**, 221–225.

Meizler, A., Roddick, F. & Porter, N. 2011 A novel glass support for the immobilization and UV-activation of horseradish peroxidase for treatment of halogenated phenols. *Chemical Engineering Journal* **172**, 792–798.

Mohan, S. V., Prasad, K. K., Rao, N. C. & Sarma, P. N. 2005 Acid azo dye degradation by free and immobilized horseradish peroxidase (HRP) catalyzed process. *Chemosphere* **58**, 1097–1105.

Soares, C. M. F., Santana, M. H. A., Zanin, G. M. & Castro, H. F. 2003 Effect of poly(ethylene) glycol and albumin on the immobilization of microbial lipase and catalysis and catalysis in organic media. *Química Nova* **26**, 832–838.

Souza, S., Forgiarini, E. & de Souza, A. A. U. 2007 Toxicity of textile dyes and their degradation by the enzyme horseradish peroxidase (HRP). *Journal of Hazardous Materials* **147**, 1073–1078.

Vámos-Vigyázó, L. 1981 Polyphenol oxidase and peroxidase in fruits and vegetables. *Food Science and Nutrition* **15**, 49–127.

Veitch, N. C. 2004 Horseradish peroxidase: a modern view of a classic enzyme. *Phytochemistry* **65**, 249–259.

Zanoni, M. V. & Carneiro, P. A. 2001 Disposal of dyes. *Ciência Hoje* **29**, 61–64.

Zeraik, A. E., de Souza, F. S., Fatibello, O. & Leite, O. D. 2008 Development of a spot test for peroxidase activity monitoring during a purification procedure. *Química Nova* **31**, 731–734.

First received 30 June 2011; accepted in revised form 3 October 2011